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Attn: **8(d) Reporting**

Dear Sir or Madam:

In accordance with 40 CFR Part 716, 59 Fed. Reg. 5956-5962 (February 9, 1994) [for a total of 37 chemicals], Union Carbide Corporation ("Union Carbide") herewith submits the studies listed in Attachment I for the following chemicals:

CAS Registry No. (CASRN)

Chemical Name [Other Chemical Names Including Product Name]

86940000393	108-90-7	Chlorobenzene (benzene chloride)
86940000394	70131-67-8	Siloxanes and silicones, di-Me, hydroxy-terminated (L-9000/1000; 45-134)
86940000395		

Attachment I provides a summary of where information can be located in Attachment II for each chemical listed above. Attachment II contains copies of toxicology reports.

In most of these reports the term "CONFIDENTIAL" may appear. This precautionary statement was for internal use at the time of issuance of these reports. There is no information in these submittals for which Union Carbide

asserts a claim of confidentiality, and the Agency may use the information as necessary in the discharge of its duties. We advise the Agency, however, that the publication rights are the property of Union Carbide.

Where material is deleted from certain reports, it is because the deleted information pertains solely to chemicals other than the ones subject to this TSCA 8(d) submission.

To the best of our knowledge, the enclosed information represents all unpublished reports on the subject chemicals. If, as a result of the file search initiated to comply with these reporting requirements, any additional unpublished health and safety studies are identified, they will be promptly submitted.

Please communicate through my office with any questions regarding this submission, at 203/794-5230.

Very truly yours,



William C. Kuryla, Ph.D.
Associate Director
Product Safety

Attachments

ATTACHMENT I
Listing of Reports in Attachment II

<u>CASRN</u>	<u>Chemical Name [Other Chemical Names Including Product Names]</u>	<u>BRRC Toxicology Reports</u>	<u>Ext. Reports (I.D.No. & Date)</u>	<u>UCC Env. Tox I.D. & Date)</u>
108-90-7	Chlorobenzene (benzene chloride)	10-29	--	Exempt
70131-67-8	Siloxanes and silicones, di-Me, hydroxy-terminated (L-9000/1000; 45-134 [contains 80%])	40-77; 45-134	--	--

PART II

TOXICOLOGY

STUDIES

Confidential
Report No. 1467

INDUSTRIAL TOXICOLOGY DEPT.
FILES

11-13-47
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PELLON INSTITUTE OF INDUSTRIAL RESEARCH
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on

A Review of the Rabbit Ear Chloracne Test

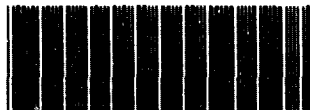
Carbide and Carbon Chemicals Corporation

Industrial Fellowship No. 274-10

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Summary

Newly made microscopic examinations of some 360 rabbit ears treated during the past 6 years convinces us that we cannot distinguish fine differences in hyperplastic response provoking abilities of samples. Work to refine the test has been planned. The data on all materials tested is presented in tabular form to agree with our present interpretation of the test.

Background and Review of the Test

In 1941 Adams, Irish, and Spencer (1) of the Dow Chemical Company published a paper about a skin reaction produced in rabbits which appeared to parallel the acneform dermatitis or chloracne seen on human skin from contact with various industrial materials. On the rabbit the reaction was an epithelial hyperplasia, with resulting thickening of the skin and enlargement of the follicles. Those workers were convinced that experimental applications of chemicals to rabbit ear or belly could be employed to evaluate the particular property of a material which enabled it to produce chloracne on the human skin. Personal communications from these authors both before and after their publication have enlarged our knowledge of their technic.

Our report 4-30, dated September 8, 1941, described their test and our own adaptation of it. We first made 17 applications of 0.5 ml. volumes of solutions of the samples during three weeks to the inner surface of rabbit ears. Due to the large volume of fluid applied, wide-spread reactions were produced, extending down over the head and neck of the animals. In this series we did establish that 5% solutions, approximately saturation of highly _____, were necessary to obtain sufficiently intense reactions, and that concentration has been considered standard for the past 6 years.

The next series employed 0.1 ml. of 5% olive oil solutions applied 30 times during 6 weeks. Since that time 30 applications has been considered standard. As new samples began to reach us we ran into difficulties due to limited solubility in _____. The oil had never been entirely satisfactory because it causes itself a mild hyperplastic response similar to that from true chloracne producers. One sample was dissolved in _____ and a few were suspended in _____.

After several series of tests had been made it became apparent that standardization upon a vehicle was desirable. We abandoned hope of applying solutions of the multiform resins and mixtures which reached us and decided that all would be applied as suspensions. The mixture selected for a vehicle consisted of 120 gm. _____ and 80 ml. of _____. This is heated to 90° C. and 5% by weight of the sample, powdered as well as possible, is stirred in, continuing stirring until the mixture is cool. The result is an homogeneous mixture which is soft and easily spread upon the ear. The vehicle alone produces no microscopic changes in the rabbit ear, but materials suspended in it cause less severe changes than when they are dissolved in oil. This is no doubt due both to poorer contact with the ear by the suspended sample and to the lower background effect from the vehicle alone.

Gross Examination

In our hands the test never yielded gross results sufficiently definite so that we felt secure in relying solely upon the appearance of the treated ear to diagnose its response, but gross observation is still the main reliance of the Dow group.

Measurement of Ear Thickness

We at first hoped that the hyperplastic changes characteristic of the rabbit response would be accurately reflected in increases in thickness of the ear, which could be evaluated by measurement on the living animal. For this reason the thickness of more than 350 ears was measured before and after treatment with a paper thickness gauge. The point of measurement was one-third of the distance from the ear tip to the skull, and about one-third of the distance from the outer to the inner edge. This is the location at which a tissue sample is taken for microscopic examination at the termination of the test. The mean thickness of the ears of our rabbits at the start of the test was found to be 0.0346 inches, with a standard deviation of ± 0.0065 inches.

During the six week period of applications the following mean increases in thickness have been observed. An asterisk (*) marks those which have a statistically significant difference from the increase produced by the vehicles alone.

Untreated control	0.006 inches
<u>Vehicles</u>	
	0.014
	0.012
	0.005
<u>Samples</u>	
	0.010*
	0.007
	0.022*
	0.012
	0.008
	0.009
	0.009
	0.012*
	0.009
	0.013

We have concluded that these increases in thickness are not sufficiently marked or constant to justify our depending upon them for diagnosis of the results of the test, and unless the technic is modified to lead to a greater increase it is not worthwhile to continue the measurement. Accordingly we have come to depend almost entirely upon study of a microscopic section of the ear for diagnosis of the degree of response, despite the fact that the Dow workers study microscopic sections only occasionally and rely entirely upon gross appearance and thickness for diagnosis. We have recently learned that they do not attempt such fine distinctions in response as we do and that they regard our

4 as only a mild chloracne producer compared to some of the which are their chief interest. The fact that

is about the most active compound we have applied helps to reconcile our necessity for microscopic examination with their dependance upon gross appearance.

Previous Pathological Interpretation

Our interpretation of pathology associated with the five stages of response which Adams et al. describe (1) was outlined in our report 4-80 and is repeated below as it was employed up to January 1947.

Stage 1. (thought not to be significant since recovery is very rapid). Surface epithelium slightly thicker than normal and may bear small projections pointed inward, consisting of only a few cells. Hair follicles somewhat broad at the top, but not raised above the surrounding epithelium.

Stage 2. Surface and follicular epithelium up to twice as thick as normal. Downward projections of epithelium larger and consisting of many cells. Hair follicles slightly raised above surrounding epithelium.

Stage 3. Surface epithelium still thicker and downward projections reach nearly to the cartilage. Follicles are raised still further.

Stage 4. Follicular epithelium spreads outward and downward and approaches or even engulfs sebaceous glands. Congestion, hemorrhages, edema, and leucocyte infiltration may be seen.

Stage 5. Keratinization with engulfed follicles and marked sloughing.

For two series of tests run in 1946 and 1947 an attempt was made to assign numerical points to each feature of micropathology and thus achieve a numerical score for each slide. The variation between animals made this impractical and the attempt has been abandoned. Accordingly the scheme will not be detailed here.

September 1947 Status

As has happened to us with other tests, we now find that circumstances have led us into pushing the interpretation of the results of the chloracne test further and further in an effort to determine finer and finer distinctions between nearly identical degrees of response. The Dow workers are satisfied with one rabbit treated with each of a small series of concentrations, but they make no attempt to draw fine distinctions between samples. We depend on only one concentration, but the variation between animals has led us to use more and more ears for one sample until now, if we used the number we feel is desirable our animal quarters could accommodate rabbits for only this one test.

The time has come to take stock of our chloracne test and to determine just what degree of difference between compounds can be detected with certainty. If this degree does not meet our needs, it will be necessary to study the condition of the test to discover if changes will increase its precision. To accomplish this stock taking the writer desired to re-examine all of the 400 odd ear sections studied earlier, a few at a time, as single samples were tested. Unfortunately, due to a short cut adopted for slide mounting and abandoned this year, these had all faded so much that they were useless. For some 360 of the ears, bits of tissue embedded in paraffin ready for sectioning on the microtome were still filed. The missing ears has been consumed in the preparation of the original slides, now faded, because our technicians have always found the

sectioning of ears difficult and many attempts to obtain useful slides had consumed the entire tissue.

Accordingly during September some 360 new slides were made from the old blocks and during the past few weeks the writer has studied these in some detail. The descriptions of microscopic appearance which follow are based entirely on this current examination, which has the advantage over previous study that the work was done during a brief period of concentrated attention and therefore the interpretations should be more consistent.

The current stock taking does not extend to the two series of tests in which we tried to evaluate the chloracne producing powers of vapors and sublimed crystals of a number of samples. We now regard these series as misconceived efforts, which we would regret to take up again.

Microscopic Appearance of the Normal Rabbit Ear

Under a magnification of 100 diameters the hematoxylin and eosin stained cross section of the normal untreated rabbit ear has quite definite characteristics. Down the center runs a wide ribbon of cartilage resembling superficially a streak of purple foam. Bounding the section upon each side is the epithelial layer, also purple. Between the cartilage and the epithelium on both sides lies a wider area of pink-stained connective and muscle tissue, with widely scattered purple-stained elongated cell nuclei. In this layer red blood cells, capillaries and larger blood vessels are distinguishable, but the most conspicuous structures are the hair follicles with their attendant clumps of sebaceous gland cells. The follicle is chiefly a slightly tapered tubular extension of the epithelial layer, projecting into the muscle tissue. The follicular epithelium appears at this magnification identical with the epithelial layer which bounds the ear. In the normal ear it hugs closely the hair shaft or multiple hair shafts, with only an indistinguishable film of lubricating sebum separating hair and follicular epithelium. The usual section contains upwards of 100 hair follicles, many in clusters. Each follicle is a tapered round tube, but they are oriented in all directions, and their sections may take many forms and a wide range of diameters. Near the edge of the ear, at the two ends of the section, hair follicles and their gland cells predominate and muscle fibers are very scarce.

The epithelial layer on the inner surface of the ear averages 20 microns in thickness, and ranges from 12 to 30 microns thick. The inner margin of this layer, actually the basement membrane, is intact, almost as if drawn with a ruler. Cell divisions within the epithelial layer are not evident, but three shapes of cell nuclei are visible with a well corrected optical system, stained a darker purple than the rest of the epithelium. They are close to the limit of resolution of the microscope at a magnification of 100 diameters. Outermost in the epithelium are one or two layers of widely spaced flattened nuclei. Inside these are one or two layers of oval nuclei closer together. Innermost, lying on the inner intact margin of the epithelium, is one close packed layer of round nuclei. In the living rabbit the entire epithelial layer is constantly being renewed as new cells form at the basement membrane and the older cells move outward, the nuclei growing flatter as they advance, and finally sloughing off of the outer edge in the form of squamous epithelial flakes.

Microscopic Appearance of Severely Injured Rabbit Ears

The most severely hyperplastic ears observed to date are bounded by an epithelial layer 60 or more microns in thickness. Its inner margin contains numerous extensions 50 or more microns wide, which may project nearly to the cartilage. Careful examination reveals the results of an intense activity at the basement membrane, by which cell multiplication has been so rapid that the normal outward rate of movement of cells cannot accommodate the newcomers. Instead of one row of round nuclei we see two, three, or four with a corresponding increase in oval and flattened nuclei. Lastly, outside the area of living epithelial cells we see sheets composed of dead keratinized cells which are still loosely attached to the outer boundary of the ear. The round nuclei of the inner margin of the epithelium often seem to be arranged in parallel filaments with a dividing line visible between them, suggesting short parallel chains of beads as if cell division had proceeded like that of the streptococci to yield cells bound into chains, each distinct and not attached to neighboring chains.

Exactly similar changes are visible in the follicular epithelium, but they often are more exaggerated here, leading to follicle walls thicker than the epithelial layer of the ear. The activity appears with severe injury to be most intense at the bottom of follicles, so that when seen in transverse section the follicular epithelium is broadest at the base of the follicle. The sheets of keratinized cells sloughed off from the inner walls of the follicle cannot leave the follicle easily. Consequently as they accumulate the lumen of the follicle is distended and the hair shaft is seen to be separated from the follicular epithelium by a mass of keratin shreds often as much as 100 microns thick. Often with severely injured ears the mass of keratin has been expelled by movement of the ear, tearing the hair from its root, and leaving a gaping hole lined with follicular epithelium.

Where several follicles are close together and would appear as a clump in a normal ear, in the injured ear their epithelia merge so that no division is evident between adjacent follicles.

In the most severely injured ears we have seen there is no change evident in the cartilage or in the sebaceous gland cells, although the latter may be completely surrounded and to some extent choked by hyperplastic epithelium. The intense activity at the basement membrane leads to a somewhat increased blood supply, and congestion of the capillaries is evident but is not judged important to diagnosis.

Some samples, notably _____, have been primary irritants and they have caused a considerable degree of edema. In the microscopic section this is seen as a separation of the muscle and connective tissue fibers by clear spaces which were filled with lymph in the living ear. This edema is not a phenomenon associated with agents provoking a maximum degree of epithelial hyperplasia and it has not been considered in diagnosis. It does affect the thickness of the ear, but it has a tendency to reach a peak during the first two or three weeks of application and to subside materially by the sixth week. Also it is easily evident grossly, the ear being boggy to the touch, not tense or springy.

Intermediate Stages

It is evident from the two foregoing descriptions that the process of epithelial hyperplasia with which we are concerned is no more than an intensification of the normal replacement of old epithelial cells by newly formed cells. The intensification is no doubt brought about by a particular degree of injury to the cell from a class of irritants, not sufficiently severe to result in necrosis but sufficiently profound so that the cells must be replaced to maintain intact the skin's protective barrier against foreign chemicals. All the visible changes described result from the one phenomenon of intensified activity of the basement membrane and they do not represent various stages in a complex series of effects. Any definition of stages in the process will necessarily be artificial and empirical.

In one series of tests biopsy specimens were taken from ears after 17 applications and after 30 applications. Then the rabbit was killed two weeks after the 30th application and a section was studied. At the 17th application a moderate hyperplastic response was seen, at the 30th a severe response, and after two weeks rest the activity had subsided materially. This indicates that the response is progressive and that it decreases after withdrawal of the irritant.

It appears that there is a maximum degree of hyperplastic response beyond which the ear cannot respond. This maximum may be reached soon with a very active chemical and later with a less active one or with a lower concentration of the first. Less active materials will never produce the maximum possible response. Conversation with the Dow group indicates that with continued application of any chemical there is a peak in response and then a decline, but our own work has not yet shown that. For the sake of having a standard technic it is necessary that the ear be examined microscopically after some constant number of applications, which in our case is 30. At that time some ears will not have attained the peak response possible for the chemical, some will have reached the maximum possible for the ear, and others will have passed the peak and have started to regress. The only way that these three conditions can be distinguished is by frequent gross observation during the course of the applications. Fortunately, the uncertainty will be greatest with the most active chemicals, and our present interests lie with those which are least active.

Detectable Degrees of Response

The writer's present study of sections leads him to recognize certain definite visible phenomena of hyperplastic response which he believes occur in the order listed below, both as a time sequence during continued application of a very active chemical and as an activity sequence with a series of chemicals. Each item implies the presence of all previous items.

- 0 Ear normal
- a. Marginal and perhaps also follicular epithelium 50% or more thicker than normal
- b. Inner edge of marginal epithelium undulate
- c. Marginal epithelium contains two rows of close packed round nuclei
- d. Parallel streamers of cells in chains project inward from inner edge of marginal epithelium
- e. Extensions several cells wide and deep project inward from inner edge of marginal epithelium
- f. Follicular epithelium is thicker than marginal epithelium
- g. Base of follicles sectioned transversely is considerably wider than mouth
- h. Clumps of follicles sectioned across are crowded or flattened
- i. Epithelium of clumps of follicles merges
- j. Follicle dilated with keratin flakes as thick as hair shaft
- k. Follicle dilated with keratin flakes as thick as follicular epithelium
- l. Follicle dilated with keratin flakes 50 or more microns thick, or dilated follicle empty

The above listing of distinguishable phenomena in response does not constitute a numerical scale, and an ear which has attained response "l" has responded more but not necessarily twice as much as another ear with response "f". It is the writer's view that responses a, b, c, and d are mild, and we now call an ear showing them as in Stage I. Stage II, moderate response, includes those showing e, f, g, h, and i. Stage III, severe response, includes j, k, and l. We do not believe the variations in the test justify distinguishing more than these three stages of response.

Our previous 5 grades or stages, described above, we now feel to be based on too much attention to the depth of the follicle in transverse section, and this depth varies with the angle of sectioning and hence is irrelevant. We also paid too little attention to the degree of follicular dilation.

Reinterpretation of Previous Tests

Table 10-298 summarizes the recent re-examination of slides for treatments with 5% suspensions in _____, and Table 10-299 for other treatments. The results are presented in the form of cumulative percentages of the rabbit ears which showed a response at least as severe as the stage indicated. From these our present views of the chloracne producing abilities of the materials tested in _____ vehicle can be obtained by inspection of the tables.

Table 10-298

Summary of Results with Present Standard Method

Material Applied	Vehicle	Concen- tration %	Original Ears Report * Studied in 1947	% which had attained each response stage, corrected for control**			
				I	II	III	
Control	Cbx	100	-	14	0	0	0
"	"	5	-	20	100	95	65
"	"	"	-	7	100	100	72
"	"	"	-	13	31	23	23
"	"	"	>H 1020	7	100	86	58
"	"	"	<H 1020	8	100	100	100
"	"	"	0/3	3	67	0	0
"	"	"	0/3	3	33	0	0
"	"	"	0/3	3	0	0	0
"	"	"	3/4	1	0	0	0
"	"	"	>H 1000	8	100	100	100
"	"	"	>H 1000	8	100	100	100
"	"	"	=H 1014	6	100	83	83
"	"	"	0/3	3	67	67	33
"	"	"	0/3	3	33	33	33
"	"	"	1/2	3	33	0	0
"	"	"	2/4	1	100	100	100
"	"	"	-	3	33	33	33
"	"	"	0/3	3	0	0	0
"	"	"	0/3	3	0	0	0
"	"	"	0/3	3	33	0	0
"	"	"	<H 1014	3	0	0	0
"	"	"	<H 1014	3	100	100	0
"	"	"	<H 1014	2	100	50	50

Table 10-299

Summary of Results with Previous Methods

Material Applied	Vehicle	Concen- tration %	Original Report *	Ears Studied in 1947	% which had attained each response stage, corrected for control**		
					I	II	III
Control	0. 011	100	-	11	73	64	27
"	"	5	1/7	10	27	36	43
"	"	"	2/3	3	27	36	73
"	"	"	6/9	10	27	16	43
"	"	"	1/2	2	27	36	73
"	"	"	3/4	3	27	36	73
"	"	"	2/3	3	27	36	73
"	"	"	1/1	1	27	36	73
"	"	"	0/3	3	27	36	6
"	"	"	0/3	3	<	<	<
"	"	"	0/3	3	<	<	<
"	"	"	0/4	4	27	<	6
"	"	"	1/4	4	27	36	73
"	"	"	2/4	3	27	36	73
"	"	"	1/4	4	27	36	73
"	"	"	0/3	3	27	<	<
"	"	"	0/3	3	27	36	73
"	"	"	0/3	2	<	<	<
"	"	"	0/3	1	27	<	<
"	"	"	0/3	1	27	<	<
"	"	"	6/23	18	16	8	40
"	"	"	2/5	5	7	16	53
"	"	"	0/1	1	27	36	<
"	"	"	1/2	3	<	<	6
"	"	"	0/2	2	27	<	23
"	"	"	< 1014	5	7	16	13
"	"	"	< 1014	3	27	36	<
"	"	"	< 1014	3	27	36	73
"	"	"	0/2	2	27	<	23
"	"	"	0/2	1	27	36	73
"	"	"	1/3	3	27	<	6
"	"	"	0/3	2	27	<	<
"	"	"	0/3	2	27	<	<
"	"	"	5/6	3	27	36	73
"	"	"	0/3	2	<	<	<
Control	Lan.	100	-	6	33	33	0
"	"	5	-	3	67	33	33
"	"	"	1/3	3	33	0	33
"	"	"	0/3	4	<	<	0
"	"	"	-	7	24	9	42
"	"	"	5/6	3	67	67	100

(Continued)

Table 10-299 Continued

Material Applied	Vehicle	Concen- tration %	Original Report *	Ears Studied in 1947	% which had attained each response stage, corrected for control**		
					I	II	III
Control	-	-	-	1	0	0	100
"	"	3	2/3	3	<	<	<
"	"	1	0/3	2	<	<	<
"	"	1	2/3	3	<	<	<
Zero Control	-	-	-	11	0	0	0
Chlorobenzene	-	100	0/2	1	100	100	100

* In terms of fraction of treated animals showing Grade 2 or more severe response, or in terms of a comparison with another chemical.

** Percent of animals attaining stage of response indicated or a less advanced stage. In each case the percent of corresponding controls attaining same stages has been subtracted. The symbol < means that fewer treated animals than controls attained the given stage.

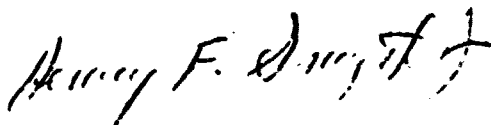
Activity of the Vehicle

With the mixture, correction for response to the vehicle alone is simple because there was no response. However, there was a very appreciable response to , as the table shows. Previously an attempt was made to correct for this during the reading of each slide. It is now realized that this practice is wrong because of the variation between animals. In Table 10-299 the correction is made by subtracting the cumulative percentages for the vehicle from those for the sample. Many times the sample gave fewer responses in a given stage than did the vehicle. The interpretation of such a result is obscure. For that reason we regard the older results with as much less useful than the more recent ones with the mixture.

Conclusions

We conclude that our technic in the chloracne test is inadequate to draw fine distinctions in the ability of a compound to elicit a hyperplastic response in the rabbit ear.

Work has been planned and some of it has been started to throw light on several uncertainties evident after reading this report.



Henry F. Smyth, Jr.

ADMINISTRATIVE FELLOW

Typed: November 11, 1947 - met

References

- (1) Adams, E. M., D. D. Irish, H. C. Spencer, Indust. Med. (Indust. Hyg. Sect. 2, 1-4, Janl. 1941). The response of rabbit skin to compounds reported to have caused acneform dermatitis.



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